CORRESPONDENCE

Bedaquiline Microheteroresistance after Cessation of Tuberculosis Treatment

TO THE EDITOR: Bedaquiline improves survival among persons with multidrug-resistant tuberculosis (MDR-TB).[1] We report the case of a 65-year-old South African man who was negative for human immunodeficiency virus and in whom MDR-TB was diagnosed in 2013 (resistant to rifampin and isoniazid; phenotypically susceptible to a fluoroquinolone and amikacin). A baseline radiograph showed changes consistent with bilateral tuberculosis with left apex cavitation. He started standardized treatment that included moxifloxacin, pyrazinamide, kanamycin, ethionamide, isoniazid, and terizidone. After initial sputum culture conversion (at month 3) and clinical improvement, the patient again became culture-positive, and bilateral cavitation developed. After detection of phenotypic resistance to fluoroquinolones (at month 6), his treatment was revised (at month 8) to include high-dose isoniazid, ethambutol, pyrazinamide, terizidone, linezolid, paraaminosalicylic acid, and kanamycin (Fig. 1 and the Supplementary Appendix, available with the full text of this letter at NEJM.org). Bedaquiline was added 22 days later and was administered for 6 months.[2] The patient remained culture-positive (treatment failure), and treatment was stopped 15 months after revision of the regimen. The patient died 7 months later.

Overall, eight *Mycobacterium tuberculosis* isolates (A through H) were assessed by means of whole-genome sequencing, targeted deep sequencing[3] of *Rv0678*, and phenotypic bedaquiline resistance testing. Whole-genome sequencing of isolate A, which was obtained 4.7 months after the initiation of standard MDR-TB treatment, revealed a Beijing strain with mutations conferring resistance to rifampin, isoniazid, ethambutol, ethionamide, fluoroquinolones, pyrazinamide, and streptomycin (Fig. 1). Whole-genome sequencing of isolate C, obtained 2 months after treatment revision, suggested that there were five potentially effective drugs in the regimen the patient had been receiving at the time that bedaquiline (to which the isolate was phenotypically susceptible) was added. Targeted deep sequencing of isolate C revealed the presence of a base-pair insertion in *Rv0678*[4] at a variant frequency of 0.05% (at position 192), indicating microheteroresistance (i.e., the presence of resistance-associated alleles at a frequency of <1%). This variant was not present in isolate B, which had been obtained before bedaquiline treatment. Isolate D, obtained after bedaquiline cessation, had this insertion in more than 90% of the bacterial population. The frequency of the insertion in *Rv0678* at position 192 decreased in subsequent isolates, but two different insertions in *Rv0678* emerged (insertion of GA at position 138 in isolate F, and insertion of G at position 138 in isolate G). The G insertion at position 138 became fixed after all treatment was stopped (iso-

THIS WEEK'S LETTERS

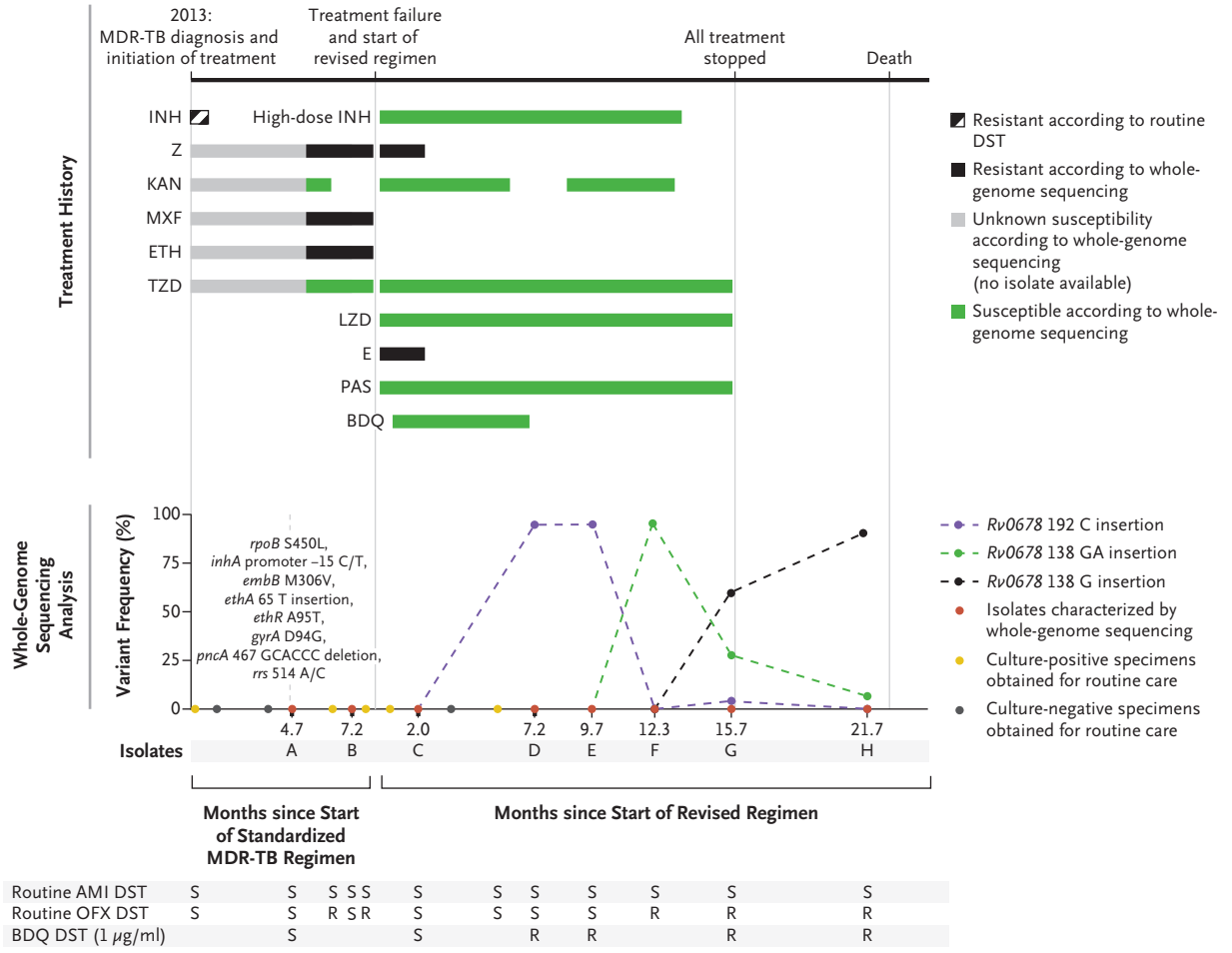

Figure 1. Chronology of Diagnosis and Treatment.

Shown is a summary of treatment, genotypic drug resistance (based on whole-genome sequencing), phenotypic bedaquiline drug-susceptibility testing (DST), targeted deep sequencing, and treatment monitoring during the standard treatment regimen and a subsequent individualized bedaquiline-containing regimen. Overall, eight isolates (A through H) collected between 4.7 months after the initiation of the standard regimen and 6 months after all tuberculosis treatment was stopped were analyzed by whole-genome sequencing, targeted deep sequencing of *Rv0678*, and phenotypic bedaquiline DST. The patient was initially given a diagnosis of multidrug-resistant tuberculosis (MDR-TB) with low-level isoniazid (INH) resistance, determined with the use of Genotype MTBDR*plus*, and was treated with a standard MDR-TB regimen. However, he remained culture-positive. In accordance with guidelines, subsequent isolates were phenotypically characterized for susceptibility to ofloxacin (OFX) and amikacin (AMI). Fluoroquinolone resistance (indicated by resistance to OFX) was first noted 6 months after treatment initiation. All isolates remained susceptible to second-line injectable antibiotics. At 8.1 months, a revised regimen was initiated, with subsequent addition of bedaquiline (BDQ) (22 days after the initiation of the revised regimen) and withdrawal of pyrazinamide (Z) and ethambutol (E) (2 months after the initiation of the revised regimen). BDQ was administered for 6 months. At month 6 of the revised regimen, the patient declined treatment with kanamycin (KAN); he continued to not receive KAN for a duration of 2.4 months. Treatment with the individualized regimen was continued until the outcome of treatment failure at 15 months. Phenotypic DST showed that all isolates with a variant frequency of more than 1% in *Rv0678* were found by mycobacteria growth indicator tube (MGIT) testing to be resistant to BDQ at a concentration of 1 μg per milliliter. ETH denotes ethionamide, LZD linezolid, MXF moxifloxacin, PAS paraaminosalicylic acid, R resistant, S susceptible, and TZD terizidone.

lates G and H). Isolates D, E, F, G, and H were phenotypically resistant to bedaquiline.

This case shows the emergence of bedaquiline resistance despite the presence of five potentially effective drugs and good adherence (based on clinical notes). The emergence of *Rv0678* variants after completion of 6 months of bedaquiline treatment shows the risk of resistance am-

plification after cessation of treatment with a drug that has a long half-life (5.5 months for bedaquiline).[5]

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